Reference: *Biol. Bull.*, **160**: 123–135. (February, 1981)

APOMICTIC PARTHENOGENESIS IN A HERMAPHRODITIC TERRESTRIAL SLUG, *DEROCERAS LAEVE* (MÜLLER)

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Abstract

Electrophoretic studies and breeding experiments show that the terrestrial pulmonate slug, *Deroceras laeve*, usually reproduces by apomictic parthenogenesis, although reciprocal outcrossing in complete hermaphrodites occurs occasionally. The sexual status of the species is controlled by the environment, with low temperatures and/or exposure to light inhibiting the development of male organs. Both hermaphrodites and females reproduce parthenogenetically. Sampling of an artificially established field population suggests that apomixis is also the rule in nature, and surveys of natural populations reveal that most populations consist of only a single clone, based on genetic identity at 20 enzyme loci. In spite of apomictic reproduction there is little heterozygosity in nature, although there is some allelic variation between populations.

Introduction

In recent years there has been renewed focus on the evolutionary implications of sexual and asexual reproduction (*e.g.*, Williams, 1975; Maynard Smith, 1978). Exploitation of both ameiotic asexual reproduction and sexual outcrossing in the same species can enhance a species' fitness (Marshall and Weir, 1979). Asexual reproduction replicates an entire genotype adapted to existing environmental conditions, favoring the expansion of a clone to the ecological limits imposed upon it. In contrast, outcrossing usually ensures genetic diversity among the offspring. In changing and unpredictable environments, sexuality provides release from genetic homogeneity, making available to selection new genetic combinations among the progeny and thereby avoiding potentially catastrophic extinction of a population by clonal elimination (*e.g.*, Shick *et al.*, 1979). A species with both modes of reproduction thus might meet all evolutionary contingencies by preserving coadapted blocks of genes in static or predictable conditions by asexual means, while allowing progressive genetic change through sexual recombination (Marshall and Weir, 1979).

Diverse modes of reproduction within hermaphroditic terrestrial slugs of the genus *Deroceras* Rafinesque (=*Agriolimax*) provide natural systems for experimental analysis of theoretical aspects of the evolutionary balance between asexual and sexual reproduction. Three species, *D. laeve* (Müller), *D. agreste* (Linnaeus), and *D. meridionale* (Reygrobellet), are capable of reproduction without outcrossing, a feature previously attributed to self-fertilization, based upon hermaphroditic morphology (Maury and Reygrobellet, 1964). These species show considerable

Received 14 July 1980, accepted 2 October 1980.

Abbreviations: CAN, TIM, BRI, SOA, PAS, MAR, MIS, HSS, BAY, MET—collection site codes for natural populations; EST1—esterase locus 1; EST2—esterase locus 2; IDH—isocitrate dehydrogenase; ME—malic enzyme; α-GPDH—α-glycerophosphate dehydrogenase.

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variation in penis morphology, with *D. laeve* sometimes entirely aphallic (Pilsbry, 1948; Reygrobellet, 1963). On the other hand, *D. reticulatum* (Müller) is an obligate outcrosser (Runham and Hunter, 1970; McCracken and Selander, 1980, and unpublished observations in our laboratory) in which hermaphroditic function is well characterized (Runham and Laryea, 1968).

In this paper we present evidence that one of these species, *Deroceras laeve*, takes advantage both of ameiotic asexual reproduction through apomictic parthenogenesis and of sexual outcrossing. Our explorations of laboratory and field populations of *D. laeve* with respect to mode of inheritance, fertility, fecundity, levels of enzyme polymorphism, and effects of rearing conditions upon sexual status suggest that this species exploits some, but perhaps not all, of the advantages available to it through the use of both modes of reproduction.

Materials and Methods

Collection and Rearing

Specimens of *Deroceras laeve* were collected from eight sites in and around Pittsburgh, Pennsylvania, and two in Canton, Ohio, by manual search of leaf litter and refuges such as stones and logs. Each site was given a three letter code; populations will be referred to by these codes. CAN and TIM are in Canton, Ohio; BRI is near Powdermill Nature Reserve, near Pittsburgh, Pennsylvania; SOA and PAS, in the vicinity of Ligoneer, PA; MAR from South Park, near Pittsburgh; MIS, HSS, BAY and MET in Mt. Lebanon, Pennsylvania.

Slugs were maintained in the laboratory on a modified slug diet (A. Gelperin, Princeton Univ., pers. comm.): a mixture of ground Purina Lab Chow, calcium carbonate, and vitamins (Vionate Vitamin-Mineral Powder for Pets, E. R. Squibb and Sons). Mass cultures of about 50 slugs were kept in plastic boxes lined with damp paper towels, with crumpled towels as refuges and oviposition sites. Clutches of eggs and small individuals were kept in 5.0 × 1.5 cm plastic Petri dishes; larger individuals were kept in small jars. Containers were changed every second day. Slugs were kept in darkness in drawers or in rooms with controlled photoperiods at either room temperature (22° ± 2°C) or 15° ± 2°C. Details of rearing conditions are given below as appropriate to the experiment.

Sexual Classification

To assess sexual status, we dissected slugs under a dissecting microscope. Each slug was classified as one of three morphological types: complete hermaphrodite, with well developed penis, prostate gland, spermathecum, and common duct; female, with penis and associated retractor muscles absent and prostate gland reduced or absent; or androgynous female, with penis reduced to a small bulge or nubbin. Pilsbry (1948) contains photographs of these variations in genitalia of *Deroceras laeve*.

Electrophoresis

Horizontal starch gels (13% W/V, Sigma) were cast in Plexiglas molds (0.8 × 12 × 20 cm) with covers and slot formers making wells of about 25 μl. Slugs were weighed and homogenized in a four-fold (W/V) volume of 0.05 *M* Tris-HCl, pH 7.1. Cutting small pieces from the posterior of large slugs did not affect the slugs' survival. Homogenates were centrifuged at 2600 × *g* for 20 min in a refrig-

erated Sorvall RC-5B centrifuge. After electrophoresis at 30 mA per gel for 4–5 h, gels were sliced and stained according to procedures modified from Brewer (1970), Selander *et al.* (1971), and Shaw and Prasad (1970). Twenty loci from 17 enzyme systems were scored using three buffer systems (Table I).

Genetic and morphological analysis of progeny

Initial experiments rearing slugs in complete isolation from egg to adult demonstrated that *D. laeve* could reproduce without outcrossing. To establish whether reproduction in isolation is due to selfing (or some other process involving recombination) or to apomictic parthenogenesis, we performed a series of breeding experiments using esterase loci (EST1 and EST2) as markers. Heterozygous strains were constructed for this purpose, since segregation cannot be detected in homozygotes without cytogenetic analysis. Ameiotic parthenogenesis is indicated if all offspring of an isolated heterozygote are likewise heterozygous. Meiosis is indicated by segregation of homozygotes and heterozygotes among progeny from such a parent.

Initially slugs from three natural populations (site codes BRI, PAS, SOA) were used to confirm reproduction without mating. Their offspring were reared in isolation from eggs to egg-laying adulthood. Pair matings were then made between homozygotes for different esterase alleles, rearing slugs in pairs from hatching to

TABLE I

Buffer systems used in electrophoresis of enzymes in Deroceras laeve. *Under each system are listed those enzymes with the number of loci for which acceptable resolution was obtained.*

Buffer system	# Loci
I. 0.1 *M* Tris-0.031 *M* Citrate, pH 7 Diluted 1:10 for gel. (Nichols and Ruddle, 1973)	
Hydroxybutyrate dehydrogenase (HBDH)	1
Isocitrate dehydrogenase (IDH)	1*
Malate dehydrogenase (MDH)	2
6-Phosphogluconate dehydrogenase (6PGDH)	1
Leucine aminopeptidase (LAP)	1
Malic enzyme (ME)	1*
Phosphoglucomutase (PGM)	1
II. Discontinuous Lithium Hydroxide (Selander *et al.*, 1971)	
β-naphthyl esterase (EST)	3*
αGlycerophosphate dehydrogenase (αGPDH)	1*
Aldehyde oxidase (AO)	1
"Tetrazolium oxidase" (TO)	1
III. Discontinuous tris citrate (Poulik) (Selander *et al.*, 1971)	
Glucose-6-phosphate dehydrogenase (G6PDH)	1
Xanthine dehydrogenase (XDH)	1
Glutamate oxalacetate transaminase (GOT)	1
Glutamate pyruvate transaminase (GPT)	1
Fructokinase (FK)	1
Phosphoglucoisomerase (PGI)	1

* Variable loci. All three esterase loci are variable.

adulthood. Three matings were made in duplicate involving the BRI, PAS, and SOA genotypes. The crosses were constructed so that progeny resulting from outcrossing were expected to be heterozygotes for either EST1, EST2, or both. EST1 heterozygotes are three-banded; EST2 heterozygotes are two-banded. BRI × SOA were expected to produce heterozygotes for EST2; PAS × SOA were expected to produce heterozygotes for EST1; while BRI × PAS were expected to produce heterozygotes at both esterase loci. One member of the second BRI × SOA pair died; the other was discarded, leaving five pairs in the experiment. Mated slugs were separated after several clutches had been laid and were allowed to continue to lay eggs for an additional 4 weeks. At that time parents were dissected and genotyped with samples of their offspring.

One large egg clutch was selected from each of the five matings. From each clutch 10 slugs, all of which proved heterozygous at the expected loci, were reared singly from hatching. Remaining clutchmates (also heterozygous) were reared in groups of five to fifteen, depending upon original clutch size. Except for one clutch, all of these slugs were reared from hatching on a controlled photoperiod of 12 h light: 12 h dark (LD 12:12) at 22°C. An exceptional clutch (BRI × SOA) was laid and thus hatched 5 days before parallel clutches from other matings. These animals were kept in darkness (LD 0:24, 22°C) until other clutches hatched, at which time all were moved to LD 12:12 at 22°C. Eggs were collected from isolated slugs and from siblings reared in groups. Each parent was dissected and genotyped electrophoretically with a sample of 15 of its newly hatched offspring.

Effects of rearing conditions on penis morphology

Deroceras laeve was reared from newly laid eggs (<24 h old) to adulthood under various combinations of pre- and post-hatch conditions to measure environmental effects on development of male organs. Four regimens were used: LD 0:24 at 22°C; LD 0:24 at 15°C; LD 12:12 at 22°C; LD 24:0 at 15°C. At hatching some groups of slugs from the four pre-hatching conditions were moved to the other regimens, so development continued under different conditions. This procedure allowed some discrimination of the sensitive stages of the life cycle. These 12 groups were supplemented with four in which slugs were reared from egg to adulthood under each of the four constant regimens. Each of the 16 experimental groups consisted of 25 slugs derived from the BRI × SOA strain and 25 from BRI × PAS. These genotypes, constructed as described above, were reared separately to assess genotype effects on morphology.

Adult slugs (≥0.1 g) were dissected and scored for sexual condition. Gonads and spermatheca from five individuals of each genotype in each of the 16 groups were stained with aceto-orcein using the method of Humason (1972), squashed, and examined under a compound microscope for gametes.

Sexual status and structure of an artificial field population

A population of *Deroceras laeve* was established in the field from laboratory stocks in September 1978 at a site previously free of slugs. One hundred homozygous newly hatched slugs were released, half with an electrophoretically fast allele for isocitrate dehydrogenase (IDH) and half with a slower allele. The population was subsequently sampled at 4 week intervals from the next April (1979) through November by collecting all adults found by manual search in 2 h. Slugs of all samples were dissected for penis morphology. All samples but November's were

genotyped for IDH to assess frequency of outcrossing. In addition, collections of at least 30 slugs were dissected in April, June, and August 1980. Gonads and spermatheca were searched for sperm or evidence of spermatogenesis by the above method.

Results

Fertility and fecundity in the laboratory

Embryogenesis requires about 14 days at room temperature (22° ± 2°C). Hatching of eggs is synchronous, typically occurring within 1–2 h for a given clutch. *Deroceras laeve* begins to lay eggs 4 weeks after hatching, at a body weight of 0.06–0.1 g. Minimum generation time for this species is thus 6 weeks. These slugs continue to lay eggs at 2–3 day intervals as long as they live, which usually is 8 weeks post-hatching but can be as long as 13 weeks. Animals continue to increase in size throughout their lives; maximum size of a laboratory reared *D. laeve* was 0.51 g, compared to 0.43 g as a maximum that we found in nature. Clutch weight and size are proportional to the parents' body weight. Mean clutch size was 18 ± 8.5 (SE) eggs. Individuals laid as many as 350 eggs over their life spans.

Fertility in *Deroceras laeve* is usually above 98%, either in terms of the number of slugs producing eggs or in the number of eggs developing per clutch. Occasional clutches are completely infertile, especially small slugs' first clutches. Fertile adults collected from natural populations throughout the warm season readily laid eggs under laboratory conditions. Field populations consist of individuals of all sizes, reflecting continuous reproduction.

Rarely, in clutches of large eggs, multiple embryos must have developed in one egg, since the number of hatched slugs exceeded the number of eggs in the clutch. The number of embryos per egg is not known.

Apomictic parthenogenesis in Deroceras laeve

Deroceras laeve reproduces both sexually by outcrossing and by apomictic asexual means. Pair matings between the homozygotes for different esterase alleles were successful and reciprocal. Both members of each pair laid fertile eggs and these progeny were all heterozygous, producing the expected mutiple banded phenotypes. Apomictic parthenogenesis was confirmed in the next generation. Of 50 isolated slugs, 43 laid fertile eggs. From these, 23 parent-offspring comparisons were made. Each of the 15 tested offspring of all unmated heterozygotes were themselves heterozygous. This complete lack of segregation is consistent only with ameiotic parthenogenesis and not with self-fertilization. The binomial probability of obtaining this result in any family of 15 segregating progeny is 3×10^{-5}. We obtained this same result in 23 families. To our surprise, all sibs reared in groups were also parthenogenetic with one exception discussed below.

Parthenogenesis is also supported by gross internal morphology. We dissected 40 slugs reared in isolation and 60 reared in groups from hatching under LD 12:12 at 22°C. These animals were entirely aphallic, yet all isolated slugs reproduced, making self-fertilization highly improbable. The female reproductive system was complete, including a spermathecum. We did not search the reproductive tract for sperm. Mated slugs, reared under LD 0:24 at 22°C, were all hermaphrodites.

The exceptional clutch (above) differed with respect to both reproductive success and internal morphology. This clutch, produced by the mating BRI × SOA, was kept under LD 0:24, 22°C for 5 days after hatching, then transferred to LD

12:12, as described in Methods. Only three of the ten isolated slugs from this clutch laid eggs, and they laid 2 weeks later than parallel isolates from other clutches. Progeny from these isolates were uniformly heterozygous and hence produced parthenogenetically. The remaining 14 slugs from the exceptional clutch, reared together, were also slow to produce eggs. Seven individual egg clutches from this mass clutch consisted entirely of nonsegregating progeny (heterozygotes). The eighth clutch of 18 eggs gave rise to three electrophoretically fast homozygotes, ten heterozygotes, and five slow homozygotes. This result is not significantly different from the expected 1:2:1 ratio from a mating between heterozygotes ($P > 0.5$). We think it unlikely that this clutch was produced by self-fertilization since no sibling in isolation (indeed no slug in this study) selfed. We did not determine which among the 14 were laying eggs, although the segregating progeny was probably produced by only one, since all came from the same egg clutch.

Of the 24 members of the initial exceptional clutch with delayed oviposition, 20 were hermaphrodites, including all 10 slugs reared in isolation. Four were female. Parthenogenesis thus occurs in hermaphrodites, as well as in females, although parthenogenetic reproduction seems to be delayed in hermaphrodites. To our knowledge, only hermaphrodites mate.

Effects of rearing conditions on penis morphology

Differences in penis morphology among lab reared *Deroceras laeve* during breeding experiments suggested that exploration of effects of photoperiod and temperature on development of male end organs might be profitable. Asexually produced slugs of two hybrid strains, BRI × SOA and BRI × PAS, were used. With one exception, there were no significant differences in resulting phenotype frequencies between the two genotypes ($P > 0.05$, G test; Sokal and Rohlf, 1969). The exception involved slugs with embryonic development under LD 0:24 at 22°C and post-hatching conditions of LD 0:24 at 15°C. Twenty BRI × SOA hybrids became hermaphrodites; five were androgynous females. Only four of 25 BRI × PAS slugs were hermaphroditic; the remainder showed incomplete development of the penis (androgynous) ($P < 0.001$). No females were found in either group. Since this is the only exception and because we do not understand its basis, we pooled all data from the two genotypes.

Figure 1 summarizes the results of experiments where eggs developed under LD 0:24 at 22°C. Slugs reared from egg to adulthood under those conditions became hermaphrodites. The frequency of complete development of male organs was decreased by more than half by moving slugs to 15°C at hatching, regardless of photoperiod. These slugs tended to become androgynous females; only four females were found in the post-hatching group LD 24:0 at 15°C. Cooler temperatures, regardless of photoperiod, seem to inhibit completion of male organs. The frequency of hermaphrodites was also decreased by exposing slugs to 12 or 24 h of light per day. All slugs moved to LD 12:12 at hatching showed at least partial development of male parts. However, only four of the 200 eggs that developed under LD 0:24 at 22°C gave rise to females; the rest showed some male development.

Embryogenesis under the other three conditions resulted in a high frequency of females and almost no complete hermaphrodites (Figs. 2–4). Eggs developing under LD 0:24 at 15°C gave rise to a small proportion of androgynous females in four post-hatching groups, but no hermaphrodites occurred, regardless of post-hatching experience (Fig. 2). Eggs developing at the warmer temperature and exposed to a day length of 12 h also tended to have incomplete or no development

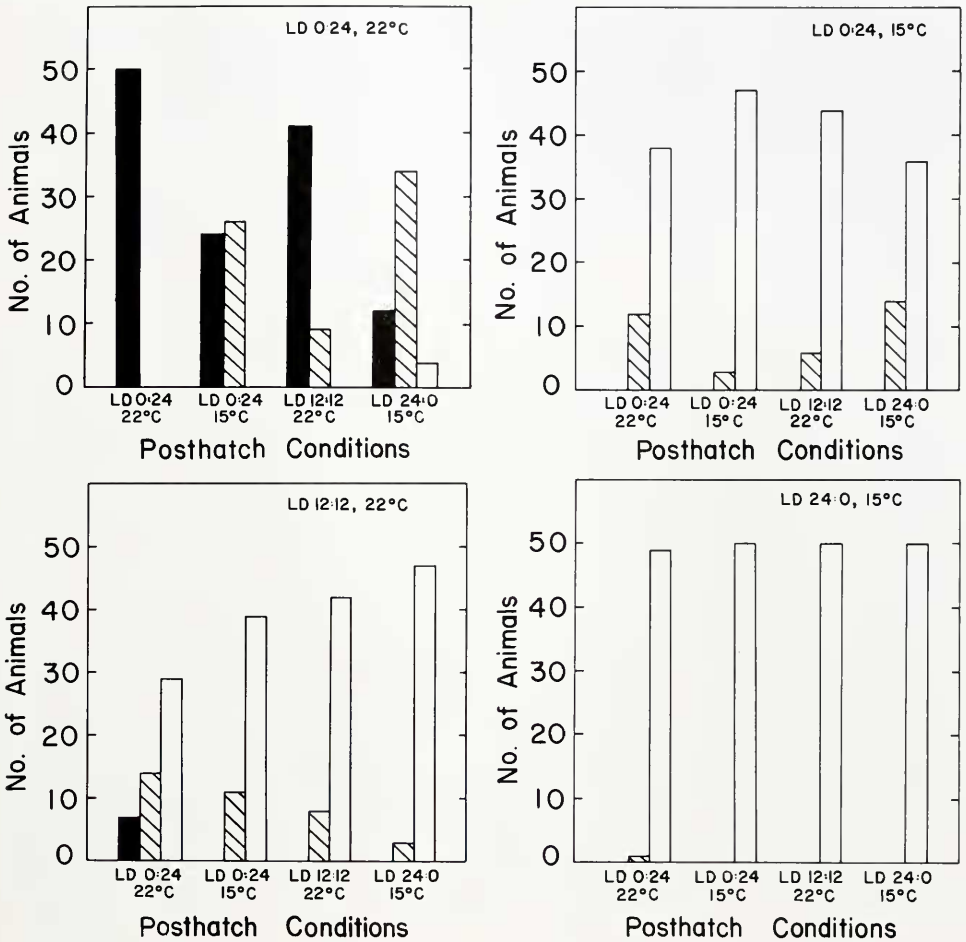

FIGURE 1. (Top left.) Frequencies of three sexual morphs among *Deroceras laeve* exposed to LD 0:24 at 22°C during embryogenesis. At hatching slugs were transferred to the indicated conditions and scored for sexual morphology as adults. Solid bars, hermaphrodites; cross-hatched bars, androgynous females; open bars, females.

FIGURE 2. (Top right.) Frequencies of sexual morphs among *Deroceras laeve* exposed to LD 0:24 at 15°C during embryogenesis and transferred to the indicated conditions at hatching.

FIGURE 3. (Below left.) Frequencies of sexual morphs among *Deroceras laeve* exposed to LD 12:12 at 22°C during embryogenesis and transferred to the indicated conditions at hatching.

FIGURE 4. (Below right.) Frequencies of sexual morphs among *Deroceras laeve* exposed to LD 24:0 at 15°C during embryogenesis and transferred to the indicated conditions at hatching.

of male parts (Fig. 3). Seven hermaphrodites were produced, all of these in the post-hatch group under LD 0:24 at 22°C. Embryonic exposure to light also inhibits development of the penis, as does low temperature. In the last set of eggs (Fig. 4) held under LD 24:0 at 15°C, only one individual showed any sign of male parts, again among slugs reared post-hatching in the dark at 22°C. The remaining 199 females in that set reflect the combined effects of cooler temperature and exposure to light in the inhibition of complete hermaphroditism in laboratory reared *Deroceras laeve*. The general picture suggests that exposure to cool temperatures and

light inhibits the development of male organs. Environmental conditions before hatching have the greater effect on adult penis morphology, but post-hatching conditions slightly modify the number of hermaphrodites among eggs developed at warmer temperatures (22°C) (Figs. 1, 3). It is interesting that in these experiments genetically identical individuals exhibited a range of sexual morphologies even when reared in the same container.

Although gonads and spermatheca of ten individuals in each rearing group were inspected under a compound microscope, no evidence of spermatogenesis nor sperm storage was found. Oocytes were common in the gonads of all three morphological types.

Sexual change in a field population

Penis morphology of *Deroceras laeve* varied during the warm season of 1979 in the artificially established field population (Fig. 5). The frequency of females was near 100% until a precipitous decline to 16% in July. In that sample 59% of the adults were hermaphrodites and 25% androgynous females. The number of females increased gradually during the remainder of the season, reaching 50% in November. Androgynous females were found at a low frequency (<25%) in every sample. Samples from this population reflect egg development 4–6 weeks previously. In general, an increasing frequency of hermaphrodites as summer progressed correlates with laboratory experiments on penis morphology: Females developed from eggs laid when temperatures were lower; male parts were produced during warmer weather. The importance of other environmental variables in nature is unknown.

No genetic evidence of outcrossing was found in samples of this population. All individuals were homozygous, with both marker alleles of IDH represented in each sample. While persistence of homozygosity could suggest self-fertilization, by analogy with the laboratory experiments, we think it more likely that it suggests continued asexual reproduction. In support of this, we were unable to detect any evidence of sperm or spermatogenesis in the 1980 samples, even though we have had no problems in visualizing them in *D. reticulatum* by the same technique

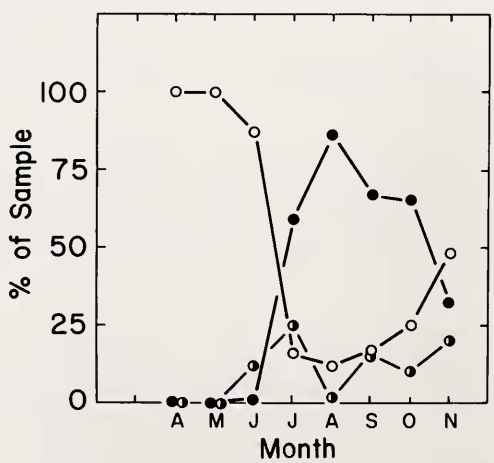

FIGURE 5. Frequencies of three sexual morphs at various times of the year among *Deroceras laeve* in an artificially established field population. Collections were made in 1979. Closed circles, hermaphrodites; half-filled circles, androgynous females; open circles, females.

(unpublished). No obvious relationship was seen between IDH genotype and sexual phenotype. Frequencies of marker alleles were similar among the three phenotypes.

Population size remained relatively stable throughout the season. Numbers of adults (≥0.06 g) discovered in 2 h by manual search of refuges ranged from a low of 48 in April to a high of 69 in June. During each collection period, small slugs and clutches of eggs were found but were left relatively undisturbed.

Electrophoretic analysis of natural populations

Laboratory studies of the mode of inheritance were supplemented with a survey of 10 natural populations of *Deroceras laeve*. Twenty electrophoretic loci of 17 enzymes were scored (Table I). Within eight populations there was no variation in banding pattern; every individual was homozygous for all loci. In one sample (site code CAN) 17 of 52 slugs were heterozygous for EST2 with a two banded phenotype. The remainder were homozygous for the fast allele. This was the only population consisting of more than one clone. From another site one mile distant (TIM) all 30 slugs collected were heterozygous at that locus. These samples were otherwise electrophoretically identical, with homozygosity at all other loci.

Among 10 populations, seven different genotypes were found involving mobility differences at isocitrate dehydrogenase (IDH), malic enzyme (ME), α-glycerophosphate dehydrophase (α-GPDH), and three esterase loci (Table II). Three alleles were found for EST2; the other variable loci had two alleles. Differences between populations generally increased with geographical distance. Genotypes I and II co-occurred at CAN as described above, differing only at EST2. Four populations about one mile distant from each other (Mt. Lebanon, Pa.) were identical at all loci scored (genotype VII). The remaining populations were more than 10 miles apart, and differed from each other at 1–6 loci. Genotype VI was the least similar to any other, differing from the others at 3–6 loci. It also contained alleles for EST2 and IDH not found in any other population. The average number of alleles held in common between populations among the 20 loci studied was 17 (85%), a relatively high degree of similarity compared with other invertebrates (Lewontin, 1974).

TABLE II

Genotypes for the six variable enzyme loci found in 10 populations of Deroceras laeve. *Alleles are named according to electrophoretic mobility, with A the most anodal. Sample sizes in parentheses.*

Genotype:	I	II	II	IV	V	VI	VII
Site code:	CAN (35)	CAN (17) TIM (30)	BRI (21)	SOA (48)	PAS (37)	MAR (62)	MIS (35) HSS (48) BAY (49) MET (50)
Locus:							
EST1	BB	BB	BB	BB	AA	AA	BB
EST2	AA	AB	AA	BB	BB	CC	BB
EST3	AA	AA	AA	AA	AA	BB	BB
IDH	AA	AA	AA	AA	AA	BB	AA
αGPDH	AA	AA	BB	BB	BB	BB	BB
ME	BB	BB	AA	AA	AA	AA	AA

Discussion

The discovery of apomictic parthenogenesis in *Deroceras laeve* represents the first documentation of parthenogenesis in a pulmonate to our knowledge. It may help to explain aspects of the slug's biology not well understood previously. *D. laeve* is believed to have migrated from Asia to North America in a post-glacial incursion (Pilsbry, 1948) and has now spread across the continent to become one of the most common slug species in non-arid regions (Pilsbry, 1948; Chichester and Getz, 1969). This species has a wide tolerance of environmental conditions relative to other species of slugs and begins to reproduce earlier in the spring than the imported *D. reticulatum* (Getz, 1959). Relatively short generation time (6 weeks from egg to egg in the laboratory), parthenogenetic reproduction, and high reproductive potential (up to 350 fertile eggs per slug) are all characteristics typical of colonizing species (Lewontin, 1965). Asexual populations of colonizing species are often characterized by sudden appearances in great numbers and abrupt disappearances, as reported for example among parthenogenetic prosobranchs, *Melanoides* spp. (Jacob, 1958a; Winterbourne, 1970) and *Potamopyrgus jenkinsi* (Jacob, 1958a; Robson, 1923), as well as in the sea anemone, *Haliplanella luciae* (Shick and Lamb, 1977). These snails and anemones are tolerant of wide temperature and salinity ranges, but mortality at the limits of tolerance is precipitous, a function of genetic uniformity. No outcrossing has been reported in these other species.

Since parthenogenesis has a potential genetic advantage over self-fertilization in preserving intact well-adapted genotypes and in avoiding the approach to homozygosity necessary with self-fertilization (Marshall and Weir, 1979), it is somewhat puzzling that most electrophoretic loci in the natural populations that we sampled were homozygous. This sort of population structure would ordinarily be expected from self-fertilization, and, in fact, has been interpreted by McCracken and Selander (1980) as indicating self-fertilization in *Deroceras laeve*, as well as a number of other slug species. Selander and Kaufman (1973) and Selander and Hudson (1976) have also interpreted a similar population structure in a land snail, *Rumina decollata*, as indicative of self-fertilization. Based only on surveys of natural populations without the breeding experiments that we report here, their interpretations seem reasonable. Nonetheless, our finding of a complete lack of segregation in 23 separate families of *D. laeve* reared from eggs laid in the absence of mates is only consistent with apomixis. No other known genetic mechanism could produce such a pattern. It is also important that one natural population that we sampled (TIM) consisted of a single clone heterozygous at the EST2 locus (Table II), a result also incompatible with self-fertilization.

In the case of the data on *D. laeve*, the species for which we have unequivocal evidence of parthenogenesis, there are some possible explanations for the incompatibility of our experimental evidence with the inferences of McCracken and Selander (1980). It is possible that the morphological species *Deroceras laeve* consists of two or more cryptic species and that we are reporting on a parthenogenetic one. Alternatively, there could be variation in reproductive mode among populations. It is also worth noting that one of the esterase loci (EST1) instrumental in our breeding studies is freeze-labile and would go undetected in the frozen samples used by McCracken and Selander (1980). Finally, McCracken and Selander (1980) did not do the breeding necessary to establish unequivocally the mating system of any of the slugs they report on, and, as they point out, they are unable to rule out parthenogenesis as a result.

The production of monogenic clones of *D. laeve* by apomixis, a process that

exactly duplicates an entire genotype, remains somewhat mysterious. Such strains should faithfully preserve any accumulated heterozygosity, even if only that produced by mutation. Our results and those of McCracken and Selander (1980) suggest that the heterozygosity expected from this mode of reproduction is not commonly present in natural populations, although we did find one such population (TIM) and a second (CAN) consisting of one monogenic clone and one heterozygous for EST2 (Table II). The genetic consequences of parthenogenesis, self-fertilization, and outcrossing are identical if all individuals in a population are genetically identical and homozygous. On the other hand, the fact that most of the 10 sampled populations in our study consisted of unique uniclonal genotypes that differ from each other may suggest that apomixis serves in this case to preserve locally well-adapted multilocus genotypes, analogous to the situation suggested for some sea anemones by Shick *et al.* (1979).

Our experiments suggest that outcrossing is infrequent in *Deroceras laeve*. None was detected in the biclonal field population constructed during the study. Laboratory stocks made with original pair matings remained heterozygous for the appropriate loci with no appearance of the homozygotes expected from sib matings, even when slugs were reared in mass culture. This suggests that no further outcrossing took place, in spite of repeated opportunity. Pair matings attempted between *D. laeve* of other populations were also unsuccessful. The slugs consistently reproduced parthenogenetically. It is possible that some populations in this study lack the ability to outcross.

The relationship between environmental conditions and development of male parts and male function must determine in part the mode of reproduction of *Deroceras laeve*. These experiments suggest that development of male end organs depends upon temperature and photoperiod. Pilsbry (1948) discusses at some length the morphological variability of natural populations of this species, with some entirely hermaphroditic, others strictly female, and still others mixed. He argues against succession of sexual phases in individuals, since only rare individuals showed partial development of the penis. Pilsbry emphasized that more attention must be paid to the season in which particular morphs are collected. The variation in sexual condition of the artificially established population throughout a season, as reported above, supports the contention that sexual morphology is primarily a result of environmental conditions. Factors leading to outcrossing in this species are unclear. Both hermaphrodites and females can be parthenogenetic. Those slugs which did mate were all hermaphrodites. Females never mated.

In other species of slugs, sexual reproduction is under endrocrinological control triggered by environmental cues, with day length of primary importance (McCrone and Sokolove, 1979; Sokolove and McCrone, 1978). Fertility and fecundity in *Deroceras reticulatum* and *Limax flavus* can be enhanced or inhibited by injection of steroid hormones (Takeda, 1979) or extracts of brain or eyestalks (Takeda, 1977). Detailed histological studies of maturation of reproductive systems in *D. reticulatum* (Runham and Laryea, 1968) and *Arion ater* (Lusis, 1966; Smith, 1966) show that light and humidity exert greater effects on spermatogenesis than oogenesis. Photoperiod is critical in male-phase maturation in *Limax maximus* but has little effect on female-phase maturation (Sokolove and McCrone, 1978). In all of these studies, development of male characteristics is more sensitive to environmental manipulation than is female expression.

Selection for rapid maturation in three species of *Deroceras* capable of reproduction without mating and most pronounced in *D. laeve* (Abeloos, 1945) may have paralled selection for parthenogenesis. Early reproduction contributes to a

high reproductive potential. Abeloos (1945) characterized development of *D. laeve* as "precocious" relative to the larger *D. agreste*, with the genus *Deroceras* in turn having fewer discrete growth phases than other limacids or arionids. Studies by Reygrobellet (1963) and Maury and Reygrobellet (1964) may suggest that *D. meridionale* and *D. agreste* are also parthenogenetic, rather than self-fertile, since in those studies discrete "clones" are described for these species as well as for *D. laeve*. Inheritance of strain-specific penis morphology was followed for 10 generations in the laboratory with no opportunity for outcrossing. Genetic analysis of the "clones" was not done, so the mode of inheritance in these species is not known.

Our discovery of both parthenogenesis and outcrossing *Deroceras laeve* extends the diversity of reproductive modes known within the class Gastropoda. Parthenogenesis has been documented in the prosobranchs *Melanoides* spp. and *Potamopyrgus jenkinsi* (Robson, 1923; Jacob, 1958a, 1958b). These species, however, are not known to outcross, consisting almost exclusively of thelytokous females which frequently are polyploid (Jacob, 1958b; Winterbourne, 1970). The majority of gastropods are sexual, but with a variety of reproductive modes. Simultaneous hermaphroditism is common among snails and slugs, *e.g., Deroceras reticulatum* (Pilsbry, 1948; Runham and Hunter, 1970). Some of these species are capable of self-fertilization, carefully documented by Ikeda (1937) for example, in the slug *Philomycus bilineatus*. It will be important to distinguish self-fertilization from parthenogenesis in other species capable of reproduction without outcrossing.

Acknowledgments

This research was supported by grants T-4 from the Health Research and Services Foundation (Pittsburgh, PA), NSF DEB77-14442, and NIH GM 25809 to R.J.H.

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